Biochemistry

NEW APPROACHES TO CROSS-LINKING HEMOGLOBIN

Suhash C. Harwani, Irfan Raziuddin, Huaping Wu, M. Paul Chiarelli and Kenneth W. Olsen*, Department of Chemistry, Loyola University Chicago, 6525 N. Sheridan Road, Chicago, IL 60626 (e-mail: sharwa@luc.edu, iraziud@luc.edu or kolsen@luc.edu)

The potential of making cross-linked hemoglobin into a blood substitute has already been established. Current methods of producing specifically cross-linked hemoglobin octamers have resulted in low yields of the desired product. The yield is low because the cross-linking reagents generally react within the hemoglobin molecule rather than between different hemoglobin tetramers. To combat this problem we making complexes of several hemoglobin molecules bound to avidin and then cross-link the different hemoglobin tetramers together. First, hemoglobin was biotinylated using NHS-SS-Biotin at a ratio that should yield one or two biotins per tetramer. Next, the biotinylated hemoglobin was passed down an avidin-agarose column and the excess biotinylated hemoglobin was washed out of the column. The bound hemoglobin was reacted with a water-soluble cross-linking reagent (BMPO3). The excess reagent was removed and the bound protein immediately react with excess fresh hemoglobin. The cross-linked product is eluted from the column by reducing the disulfide in the biotinylation reagent with β-mercaptoethanol. We are also introducing additional cross-linking sites by adding sulfhydryl groups to the protein using Traut's reagent. By adjusting the ratio of reagent to protein, reactions of hemoglobin with Traut's reagent provided a soluble product which could be cross-linked more readily with bismaleidiohexane than is possible with normal hemoglobin. The product has either intra- or inter-tetramer cross-links as demonstrated by SDS gel electrophoresis and MALDI-MS.